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TITLE: Experimental Treatment of Prostate Cancer Models with Rh2, an Isolated Ginsenoside Compound

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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Ginseng is commonly used in herbal preparations for traditional Chinese medicine. Rh2, one of the ginsenosides, has been shown to suppress growth and induce apoptosis in a number of cancer cell lines both *in vitro* and *in vivo*. To evaluate the combined efficacy of Rh2 and two chemotherapeutic agents, Taxol and mitoxantrone, mice bearing the LNCaP prostate tumor xenograft were treated with corn oil (po) and saline (iv), Rh2 (50 mg/kg po daily), Taxol (6 mg/kg iv on day 1, 4, 15 and 18), mitoxantrone (2.5 mg/kg iv on day 1, 4, 15 and 18), Rh2 + Taxol and Rh2 + mitoxantrone. Tumor volumes were measured twice weekly for 4 weeks. Serum PSA were tested using ELISA. Student t-test was performed on the data acquired and results showed statistically significant differences exist between the tumour growth ratio of control group and Rh2 + Taxol treatment group ($P<0.05$) from day 9. No statistical significant differences existed between the control group and the Rh2, Taxol or mitoxantrone monotreatment groups. Taxol monotherapy and Taxol + Rh2 combination showed significant ($p<0.05$) and very significant ($p<0.01$) inhibitory effect on serum PSA levels. Overall, our results suggest that oral administration of Rh2 can sensitize low dose of Taxol in the treatment of mice bearing subcutaneous LNCaP prostate tumors and exhibits potential as a chemosensitizer of Taxol for the treatment of prostate cancer.

14. SUBJECT TERMS:	15. NUMBER OF PAGES
LNCaP, PC-3, tumor xenografts, ginsenoside Rh2, chemotherapy	12
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Annual Summary of Research Project

Experimental Treatment of Prostate Cancer Models with Rh2, an Isolated Ginsenoside Compound
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1) Original Statement of Work (Copied from the grant proposal):

Task 1. To study the toxicity of Rh2 co-administered with Taxol or mitoxantrone in nude mice. (months 1-3)

Although our previous study showed that Rh2 is well tolerated, it is unknown whether toxicity would occur upon combination of Rh2 with conventional chemotherapeutic agents.
Thirty nude mice will be divided into 6 groups, and vehicle solution, Rh2, Taxol, mitoxantrone, Rh2 + Taxol, Rh2 + mitoxantrone will be administered.
A report will be generated by the end of the toxicity study describing any side effect(s)/toxicity. Daily observational and body weight records will be attached.

Task 2. To compare tumor inhibitory effect of Rh2 *in vivo* (months 3-19)

The efficacy of treatment with Rh2 alone and in combination with conventional therapeutic agents will be examined in the LNCaP prostate tumor model. To limit treatment groups to a manageable size, this part of study will be divided into two sections: efficacy study in androgen-dependent prostate tumor models and efficacy study in androgen-independent prostate tumor models. **Sixty** nude mice will be used in each study. These will be divided into 6 groups to which vehicle solution, Rh2, Taxol, mitoxantrone, Rh2 + Taxol, Rh2 + mitoxantrone will be administered. Tumor volume and PSA will be recorded weekly. At the end of each study, tumor tissue will be harvested and stored at -80°C until further gene expression analysis.

2) Experiment accomplished:
   a. Toxicology study:
      i. The first toxicology study was started on Jun 24, 2002 and terminated on July 10, 2002, due to the severe toxicity showed in mitoxantrone treatment group (Fig. 1). The mice in both mitoxantrone alone and mitoxantrone + Rh2 group lost >15% body weight in 1 week. Treatment was stopped and mice in both groups regained body weight.

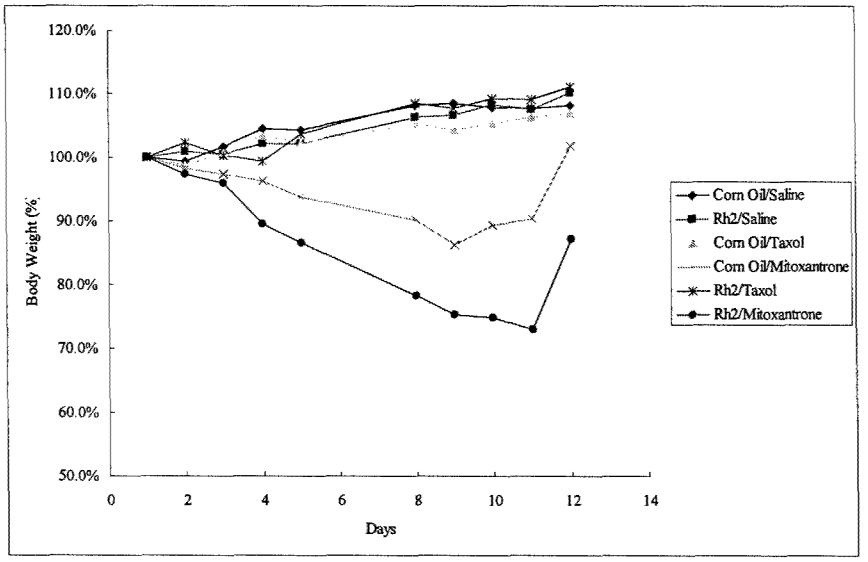

Figure 1. Body weight changes of nude mice in toxicology study.

ii. The second toxicology study was started on July 8, 2002 and terminated on August 5, 2002. Due to the mitoxantrone toxicity showed in the first toxicology study, the mitoxantrone dosage was reduced to 2.5 mg/kg i.v. twice weekly, every other week which is similar to the optimal dosage of mitoxantrone in nude mice reported by Miyake *et al* [1]. Accordingly, Taxol dosage was reduced to 6 mg/kg i.v. twice weekly, every other week.

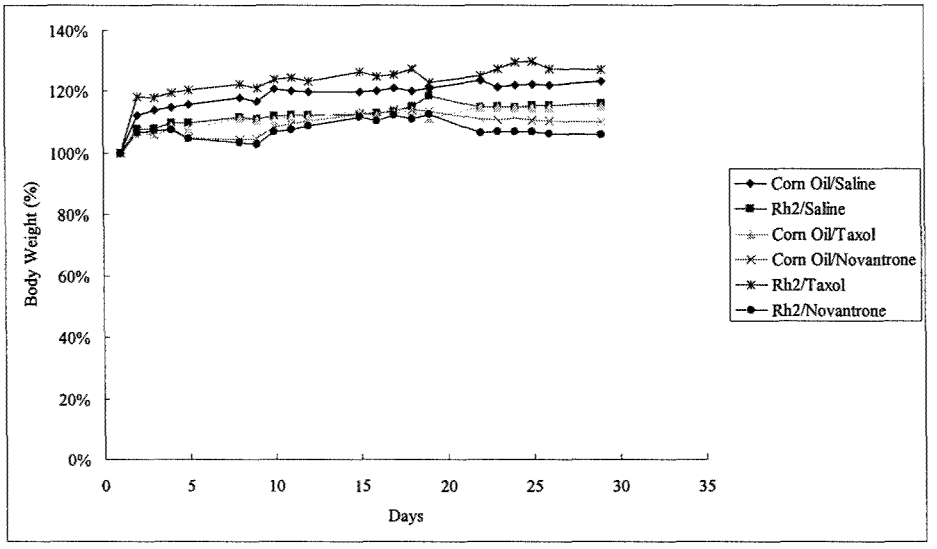

Figure 2. Body weight changes of nude mice in toxicology study

Based on the findings of the acute toxicity study, we determined that the dosing regime of 6 mg Taxol/kg body weight twice weekly and 2.5 mg mitoxantrone/kg body weight twice weekly are safe in nude mice. The change of dosing regime had been sent to Ms. Cockerham c/o Dr. Nrusingha C. Mishra on 20 June 2002 (see attached copies of email and letter).

b. Efficacy study in nude mice bearing LNCaP prostate tumour xenografts.

Sixty nude mice were purchased and inoculated with LNCaP cells. The tumours achieved 100~150 mm^3 in size by 2nd week of October 2002. The treatment started on 18 October 2002. Mice were divided randomly into 6 groups and dosed for 4 weeks as outlined in the table below.

Group	Corn Oil p.o. (5 days/week)	Rh2 50 mg/kg p.o. (5 days/week)	Saline i.v. (twice/week)	Taxol 6 mg/kg i.v. (twice/week)	Mitoxantrone 2.5 mg/kg i.v. (twice/week)
1	√		√		
2		√	√		
3	√			√	
4	√				√
5		√		√	
6		√			√

Tumour sizes were measured twice weekly and 100 µl of blood was collected via saphenous vein puncture bleeding. The serum PSA were measured using an ELISA kit (Clinpro, Union City, CA, USA).

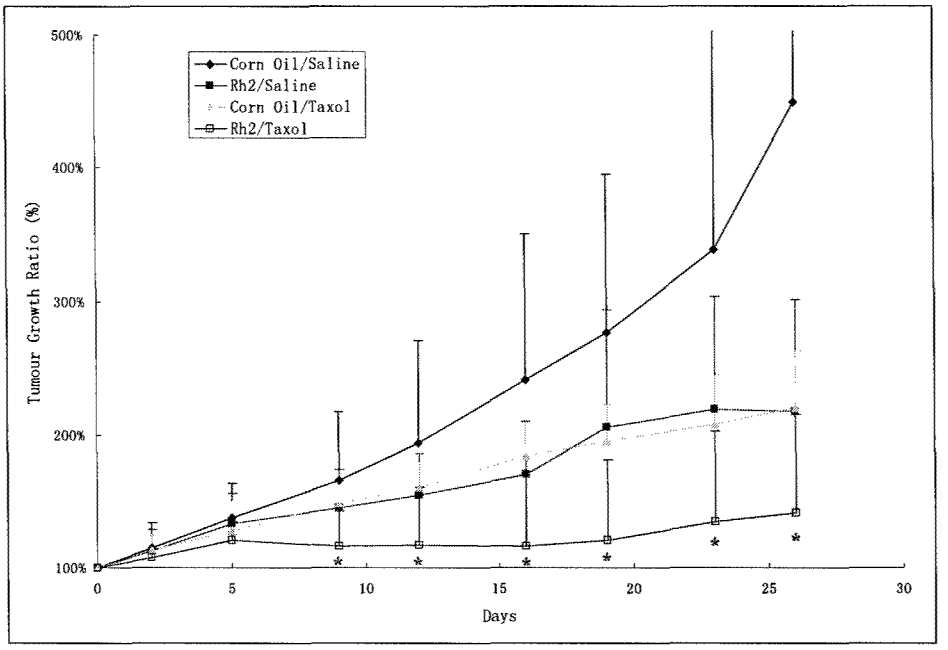

Fig. 3. Tumour growth ratio for Rh2/Taxol combination therapy. From day 9, the group treated with Rh2+Taxol showed significant suppression with control group (Student t-test, $p<0.05$*).

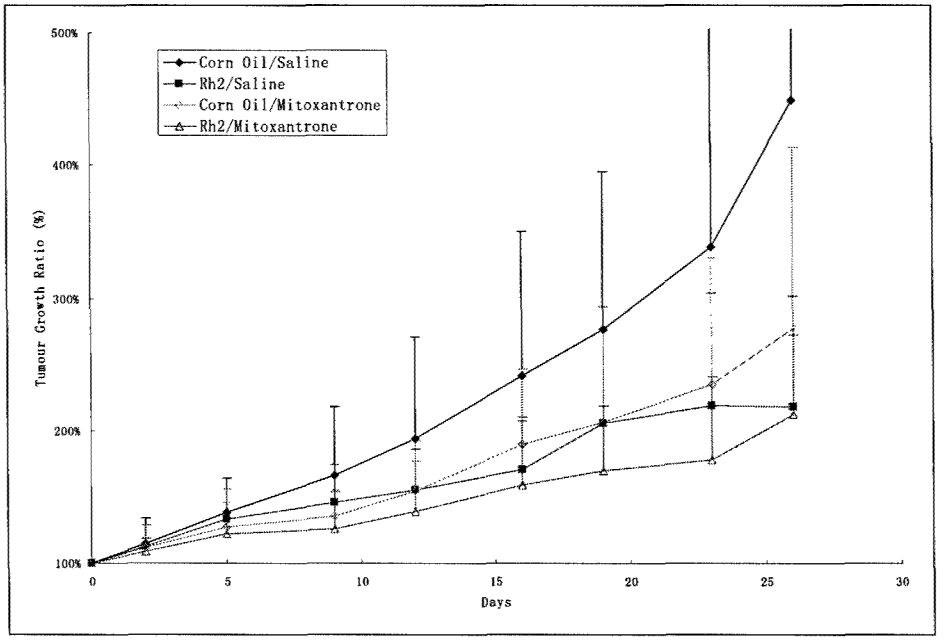

Fig. 4. Tumour growth ratio for Rh2/Mitoxantrone combination therapy

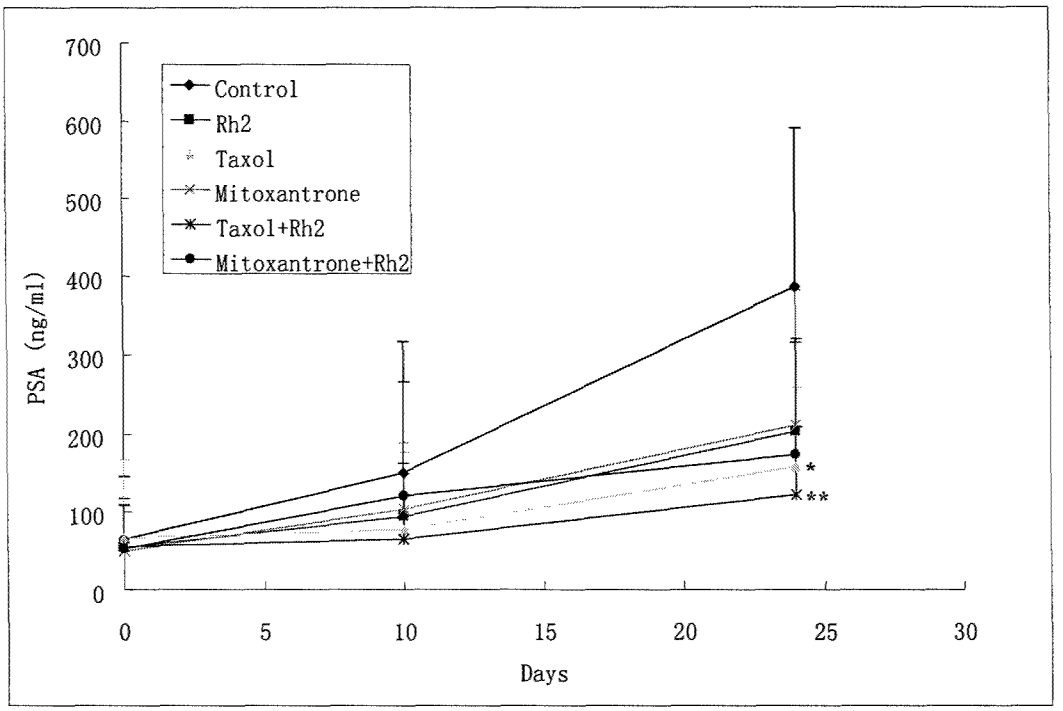

Fig. 5. The PSA value of different treatment groups. On day 24, the serum PSA of Taxol treatment group showed significant difference with that of control group (Student t-test, $p<0.05$*) and the serum PSA of Taxol+Rh2 treatment group showed very significant difference with that of control group (Student t-test, $p<0.01$**).

Key Research Accomplishments

1) Acute toxicity study showed that 4 weeks' treatment with Rh2 (50 mg/kg p.o. 5 days/week) + Taxol (6 mg/kg i.v. twice weekly) or mitoxantrone (2.5 mg/kg i.v. twice weekly) is safe for nude mice;

2) Efficacy study conducted in nude mice bearing LNCaP xenografts showed that Taxol+Rh2 treatment significantly inhibits tumour growth *in vivo* (Student t-test, $p<0.05$) and significantly inhibits serum total PSA levels (Student t-test, $p<0.01$).

Reportable Outcomes

Abstract 2699: Chemosensitization of Taxol by ginsenoside Rh2: LNCaP tumor growth suppression in vivo. 2003 Proceedings of the American Association for Cancer Research. 94th AACR Annual Meeting in Toronto, Ontario, Canada. Session EXPERIMENTAL/MOLECULAR THERAPEUTICS 25. (see attachment)

Conclusion

Low dose Taxol (6 mg/kg i.v. twice weekly) combined with ginsenoside Rh2 (50 mg/kg p.o. 5 days per week) has been proved to be safe and effective therapeutic regime in LNCaP prostate tumour models. Ginsenoside Rh2 sensitizes the tumour inhibitory effects of low dose of Taxol in this prostate tumour model.

REFERENCE

1. Miyake, H., Hara, S., Arakawa, S., Kamidono, S., and Hara, I., *Optimal timing and dosage of chemotherapy as a combined treatment with androgen withdrawal in the human prostate LNCaP tumour model.* British Journal of Cancer., 2001. **84**(6): 859-63.

From: "Mishra, Nrusingha C Dr USAMRMC" <Nrusingha.Mishra@DET.AMEDD.ARMY.MIL>

To: Sherwin Xie, UBCHGWIA.GWIA1."Wendy.Cockerham@DET.AMEDD.ARMY.MIL"

Date: Tuesday - June 18, 2002 12:34 PM

Subject: FW: US Army grant DAMD17-02-1-0260

Dr. Xie:
Please send to me your request for changes and I will forward to Ms. Cockerham. Thanks,
Nrusingha C. Mishra, Ph.D.
Grants Manager
Congressionally Directed Medical Research Programs
U.S. Army Medical Research and Materiel Command
1077 Patchel Street
Fort Detrick, Maryland 21702
Tel. 301-619-7782
Fax. 301-619-7796
e-Mail: Nrusingha.Mishra@det.amedd.army.mil

-----Original Message-----
From: Donner, Terri L Ms SAIC
Sent: Monday, June 17, 2002 1:04 PM
To: Mishra, Nrusingha C Dr USAMRMC
Subject: FW: US Army grant DAMD17-02-1-0260

For your review and response -

Teri

-----Original Message-----
From: Sherwin Xie [mailto:sxie@vanhosp.bc.ca]
Sent: Monday, June 17, 2002 12:45 PM
To: cdmrp.pa@det.amedd.army.mil
Subject: US Army grant DAMD17-02-1-0260

To whom it may concern,

I was just informed by The University of British Columbia, Research Services Department that the DoD grant money for my postdoc fellowship had been received. I want to know if there are some changes to be made in the experiment plans according to the reviewer's recommendation and our new experiment results, who should I report to? Could you give me the name of grant officer and his/her mailing address as well as fax number?

Thank you for your help.

Sherwin Xie, PhD
The Prostate Centre at Vancouver General Hospital
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Canada V6H 3Z6
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Fax: (604)875-5654

THE PROSTATE CENTRE
AT VANCOUVER GENERAL HOSPITAL

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Vancouver BC, Canada V6H 3Z6

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To: Ms. Cokerham C/O Dr. Nrusingha C. Mishra
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1077 Patchel Street
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Fax: 301-619-7796

Re: Request of change protocol for DAMD17-02-1-0260

Date: June 20, 2002

Dear Ms. Cockerham,

Please accept this letter as request of change of protocol for the postdoc trainee grant DAMD17-02-1-0260.

During our first-stage acute toxicity study in nude mice, we observed severe toxicity of mitoxantrone which caused >15% body weight loss and dehydration in 1 week. The second toxicity study showed that reduction of mitoxantrone dosage from 12 mg/kg i.v. weekly to 2.5 mg/kg i.v. twice weekly every other week and Taxol dosage (accordingly) from 12 mg/kg weekly to 6 mg/kg i.v. twice weekly is safe in nude mice.

We propose to make the following change in the efficacy study in "Task 2" in the original proposal:

Taxol dosage: 12 mg/kg i.v. weekly → 6 mg/kg i.v. twice weekly.
Mitoxantrone dosage: 12 mg/kg i.v. weekly → 2.5 mg/kg i.v. twice weekly every week.
The saline control will be used while Mitoxantrone injection is not scheduled for that week. The treatment will be given for 4 weeks (instead of 21 days) as the grant reviewers suggested.

Thank you for your consideration.

Sherwin Xiaowei Xie, PhD
The Prostate Centre at Vancouver General Hospital
2660 Oak Street
Vancouver, BC V6H 3Z6
Canada

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From: "AACR" <aacr@dbpub.com>
To: Sherwin Xie
Date: Thursday - January 23, 2003 4:41 PM
Subject: 2003 AACR Annual Meeting in Toronto, Ontario, Canada
Mime.822 (2499 bytes) [View] [Save As]

January 2003

Re: 2003 AACR Annual Meeting in Toronto, Ontario, Canada

Temporary Abstract Number #101142

Dear Dr. Xie ,

Your abstract entitled, Chemosensitization of paclitaxel by ginsenoside Rh2: LNCaP tumor growth suppression in vivo. , has been scheduled for presentation in a Poster Session at the 2003 AACR
Annual Meeting in Toronto, Ontario, Canada and will be published in the 2003 Proceedings of the American Association for Cancer Research.
Presentation information pertaining to your abstract is below:

Session ID: EXPERIMENTAL/MOLECULAR THERAPEUTICS 25
Session Date and Start Time: Monday, April 7, 8:00 AM
Permanent Abstract Number: 2699

Please refer to the printed Program or the online Annual Meeting Itinerary Planner [available through the AACR Website at http://www.aacr.org] for the exact location of your presentation. Both the printed Program and the online Itinerary Planner will be available in mid-March 2003.
The poster preparation and presentation instructions will be found on the AACR website at http://www.aacr.org in late January 2003.

Presenters at the AACR Annual Meeting must register for the full meeting at the rate appropriate to their membership status and obtain their own hotel accommodations. Forms for these purposes are available from the AACR website at http://www.aacr.org .

Sincerely,

Dr. Sara A. Courtneidge
Program Committee Chairperson

PLEASE NOTE: This document is your official notice of acceptance. No separate letter of acceptance will be mailed.

American Association for Cancer Research 2003 Annual Meeting *Abstract Proof Page*

Sherwin Xie, PhD (Refer to this abstract as # 101142)
The Prostate Centre at Vancouver General Hospital
Prostate Ctr.
JBRC 258A
2660 Oak St.
Vancouver, BC V6H 3Z6
Canada

Chemosensitization of paclitaxel by ginsenoside Rh2: LNCaP tumor growth suppression in vivo.

Xiaowei Xie, Candice Madera, William Jia, Emma Guns, The Prostate Centre at Vancouver General Hospital, Vancouver, BC, Canada; University of British Columbia, Vancouver, Canada.

Ginseng is commonly used in herbal preparations for traditional Chinese medicine. It contains more than twenty ginsenoside compounds which are the main medicinal ingredients. Rh2, a ginsenoside with a dammarane skeleton, has been shown to suppress growth and induce apoptosis in a number of cancer cell lines both in vitro and in vivo. In previous studies conducted in our lab we have demonstrated that oral administration of Rh2 (50 mg/kg) over 4 weeks has a significant growth inhibitory effect in mice bearing subcutaneously implanted LNCaP prostate tumors (Student t-test, $P<0.05$). To evaluate the combined efficacy of Rh2 and two chemotherapeutic agents, paclitaxel and mitoxantrone, mice bearing the LNCaP prostate tumor xenograft were treated with vehicle (corn oil po daily and saline iv on day 1, 4, 15 and 18), Rh2 (50 mg/kg po daily), paclitaxel (6 mg/kg iv on day 1, 4, 15 and 18), mitoxantrone (2.5 mg/kg iv on day 1, 4, 15 and 18), Rh2 + paclitaxel and Rh2 + mitoxantrone. Tumor volumes were measured twice weekly for 4 weeks. F-test was performed on data acquired for tumor growth ratio and the results indicate that statistically significant differences exist between the control group and Rh2 + paclitaxel treatment group (Tukey test, $P<0.05$) beginning on day 16. No statistical significant differences existed between the control group and the monotherapy groups (treated with Rh2, paclitaxel or mitoxantrone alone). These results correlate strongly with the synergistic results observed between Rh2 and paclitaxel in LNCaP cells in culture. No chemosensitization was observed in the group treated with Rh2 and mitoxantrone. Overall, our results suggest that oral administration of Rh2 can sensitize low dose of paclitaxel in the treatment of mice bearing subcutaneously implanted LNCaP prostate tumors and that Rh2 not only exhibits potential as a chemotherapeutic agent but also as a promising chemosensitizer of paclitaxel for the treatment of prostate cancer. (This research, under award number DAMD17-02-1-0260, was supported by the Department of Defense Prostate Cancer Research Program, which is managed by the U.S. Army Medical Research and Materiel Command)